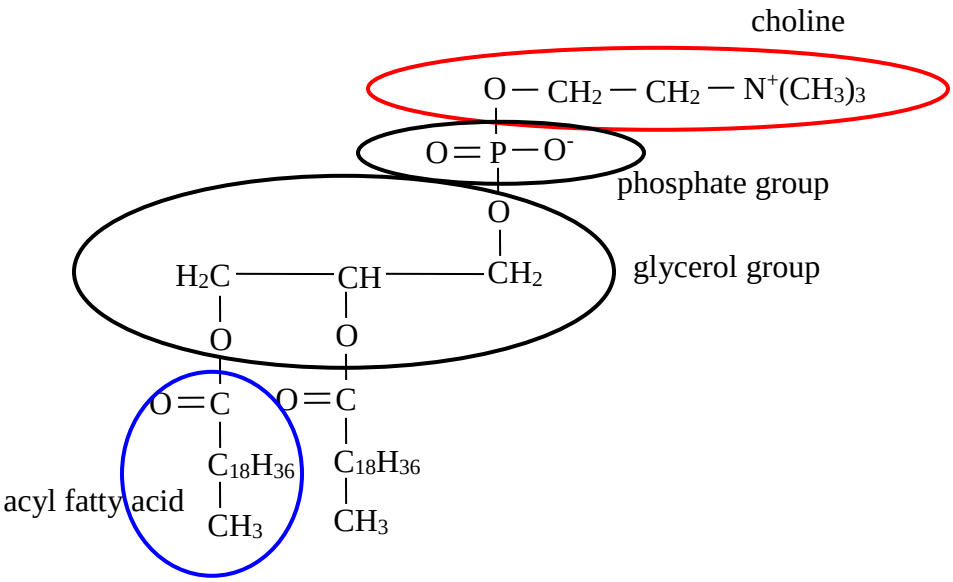
# Chapter 48 - **SANS FROM PHOSPHOLIPID BILAYERS UNDER PRESSURE**

Membranes are essential biological components for the integrity of cells and cell constituents. They create a barrier between the outside and the inside and regulate the input and output processes. Cells membranes are composed of surfactant bilayers with hydrophilic head groups and hydrophobic tails. Phospholipid molecules are typical amphiphile surfactants that form biological membranes.

## 1. PHOSPHOLIPIDS

Phospholipids form a chemical homologous series with increasing hydrocarbon tail. Dimyristoylphosphatidylcholine (DMPC) has a C14 carbon tail, dipalmitoyl phosphatidylcholine (DPPC) has a C16 carbon tail, distearoylphosphatidylcholine (DSPC) has a C18 carbon tail, and diactylphosphatidylcholine (DAPC) has a C20 acyl hydrocarbon tail, etc. These are membrane lipids that contain two saturated symmetric hydrocarbon chains. The choline group is charged and acts as the surfactant head group which interfaces with water. The acyl fatty acid groups form the hydrophobic bulk of the lipid layers.

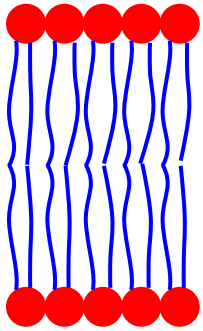



**Diacyl-phosphatidylcholine (DAPC)**

Figure 1: The various groups that form DAPC.

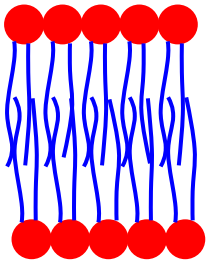
## 2. PHOSPHOLIPID BILAYERS PHASES

Phospholipids form smectic phase bilayers in the presence of water. The bilayers are formed of alternating hydrophilic and hydrophobic layers. Many phases have been identified using optical and diffraction methods. Some of these phases are: the liquid crystalline phase ($L_\alpha$) obtained at high temperature, the ripple gel phase ($P_\beta$) obtained at intermediate temperature, the lamellar gel phase ($L_\beta$) obtained at low temperature, and the lamellar crystalline phase ($L_c$) obtained at even lower temperature. Note that similar primed phases such as $L_{\beta'}$ and $P_{\beta'}$ correspond to tilted hydrocarbon tails forming the structures. A variation of the $L_\beta$ phase is the interdigitated phase ($L_{\beta I}$).



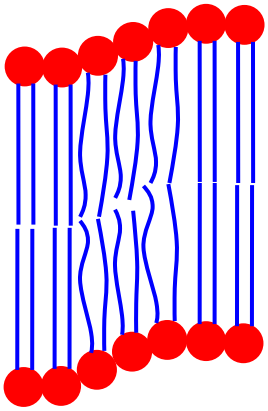
**$L_\beta$**

Lamellar gel phase



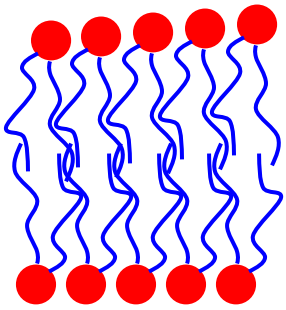
**$L_{\beta I}$**

Interdigitated phase



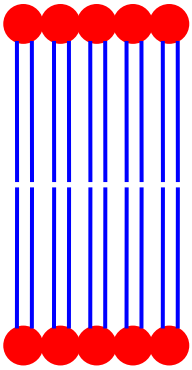
**$P_\beta$**

Ripple phase



**$L_\alpha$**

Liquid crystalline phase



**$L_c$**

Lamellar crystalline phase

Figure 2: The main phospholipid bilayer phases.

The interdigitated phase is induced by a number of factors including (1) small amphiphilic (cosurfactant) molecules such as ethanol, ethylene glycol, etc and (2) pressure. The effect of hydrostatic pressure is described here when used in conjunction with small-angle neutron scattering (SANS).

## 3. SANS UNDER PRESSURE

The SANS technique was used to investigate the transitions between the various bilayer phases in DAPC (characterized by C20) and with in-situ pressure and temperature control (Worcester-Hammouda, 1997). The sample contained about 2 % DAPC (mass fraction) in d-water and was formed of multi-lamellar vesicles. Pressure was varied up to 1 kbar (14.7 kpsi) and temperature was varied from 50 $^{o}$C to 85 $^{o}$C. A figure shows the case corresponding to 70 $^{o}$C. The liquid crystalline phase $L_{\alpha}$, the ripple gel phase $P_{\beta}$ and the interdigitated phase $L_{\beta I}$ are clearly observed. They are identified by their widely different structural features.

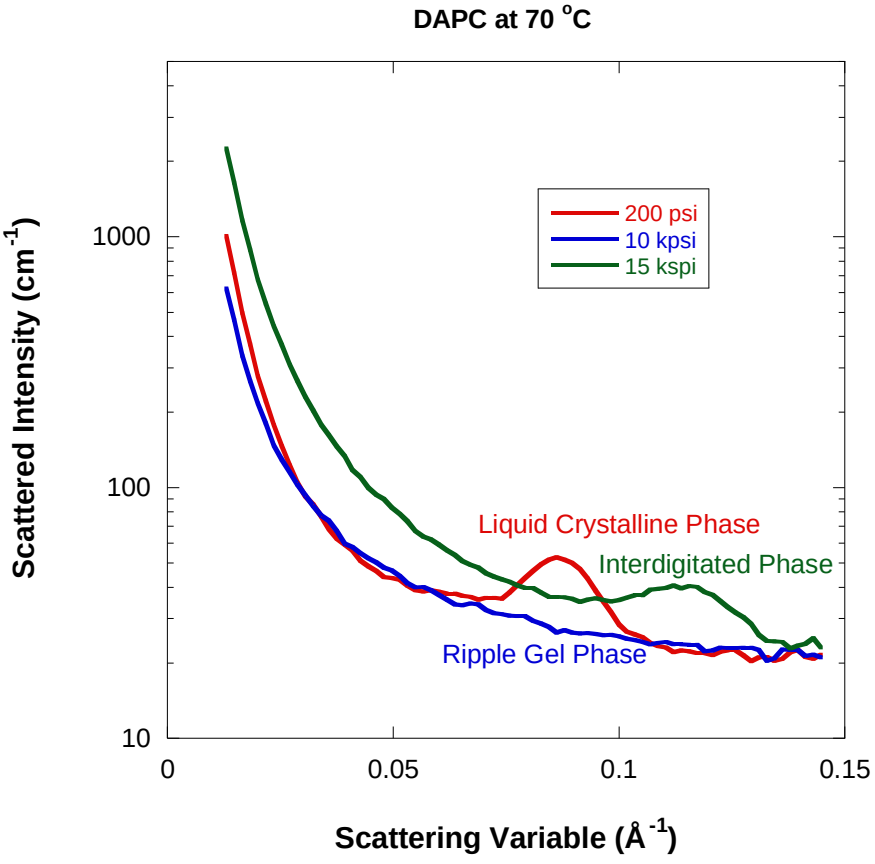



Figure 3: SANS from DAPC phospholipid bilayers at 70 $^{o}$C. Increasing pressure induces structural phase transitions.

The following peak positions are obtained for the $P_\alpha$ and $L_{\beta I}$ spectra $Q_0 = 0.085$ Å$^{-1}$ and $Q_0 = 0.114$ Å$^{-1}$ respectively. These correspond to inter-layer d-spacings ($= 2\pi/Q_0$) of 73.57 Å and 54.92 Å for the $P_\alpha$ and $L_{\beta I}$ phases respectively at 70 °C.

## 4. THE PHASE DIAGRAM

Using this method of identifying the various phases by their SANS diffraction pattern, an entire phase diagram has been constructed by varying pressure and temperature. The interdigitated phase forms only at high pressure.

Formation of the interdigitated phase by hydrostatic pressure is largely driven by the packing of acyl chains. This is otherwise difficult to achieve because of the large cross-sectional areas of the polar head groups. The curved phase boundary for interdigitation demonstrates complex properties for this packing.

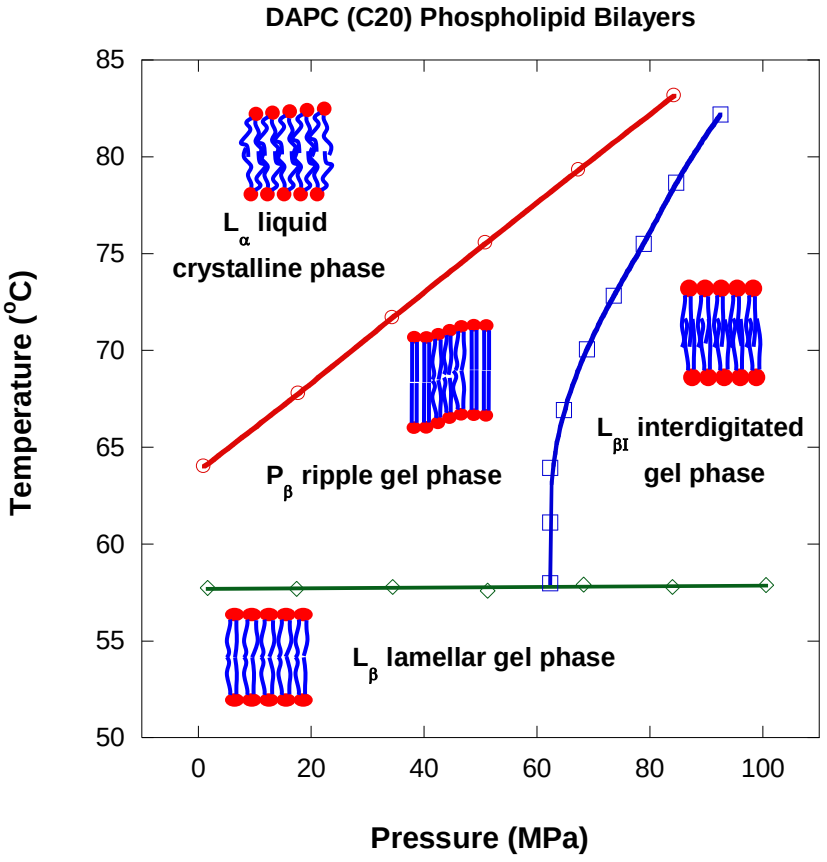



Figure 4: The temperature-pressure phase diagram for DAPC. Note that 14.7 kpsi = 1 kbar = 100 MPa.

Similar temperature-pressure phase diagrams have been mapped out for other phospholipid/d-water systems (for example DPPC and DSPC).

## 5. COMMENTS

This exercise shows once again that the SANS technique is a useful tool for resolving structures and mapping out phase boundaries. It also demonstrates the advantage of SANS measurements with in-situ pressure.

The field of membrane research has benefited from SANS as well as Neutron Reflectometry (NR). Just like SANS, NR can probe in-plane structures through the so-called off-specular scattering component.

## REFERENCES

D. Worcester and B. Hammouda, "Interdigitated Hydrocarbon Chains in C20 and C22 Phosphatidylcholines Induced by Hydrostatic Pressure", Physica B: Condensed Matter 241-243, 1175-1177 (1997).

## QUESTIONS

1. What are the major components of a biological membrane?
2. What is a surfactant?
3. What is the difference between DMPC, DPPC, DSPC and DAPC?
4. What is a smectic phase? How about a nematic phase? How about an isotopic phase?

## ANSWERS

1. A biological membrane is formed of phospholipid bilayers.
2. Phospholipids are surfactant molecules containing a hydrophilic headgroup and a hydrophobic tail.
3. The difference between the various phospholipids in the series is the length of the hydrocarbon tail; DMPC has C14, DPPC has C16, DSPC has C18 and DAPC has C20.
4. A smectic phase is a liquid crystalline phase with both translational and orientational order. A nematic phase is characterized by translational order and orientational disorder. An isotopic phase has translational and orientational disorder.